10.1071/WR23126

Wildlife Research

Supplementary Material

High chytrid prevalence and infection intensities in tadpoles of *Mixophyes fleayi*

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Appendix S1: Supplementary figures

Matthijs Hollanders, Laura F. Grogan, Hamish I. McCallum, David A. Newell

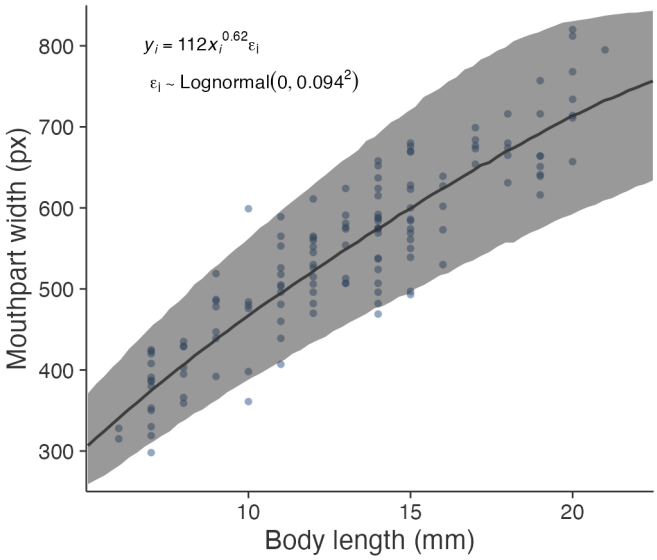

Figure S1: Scatterplot of mouthpart width as measured from photographs in pixels using Adobe Photoshop compared to body length measured with calipers in the field. The posterior predictive distribution (summarised with median and 95% HPDI) is from a log-log regression.

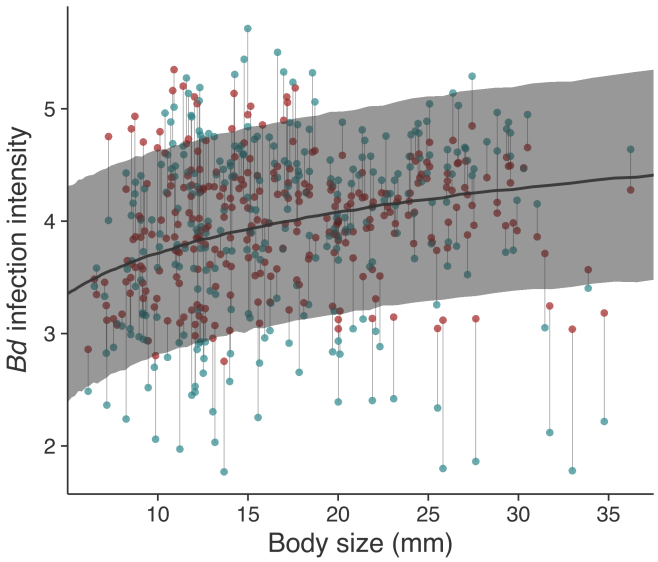

Figure S2: Posterior predictive distribution (summarised with median and 95% HPDI) for the effect of log body size (transformed from mouthpart widths using the equation in Figure S1) on *Bd* infection intensity ($\log_{10}$ gene copies per swab) of *Mixophyes fleayi* tadpoles. Blue points are observed individual *Bd* infection intensities, averaged over positive qPCR runs. Red points are posterior medians of individual infection intensities estimated by the model (m_i, Appendix S2) after propagating measurement error in the sampling (swabbing) and diagnostic (qPCR) processes.

Appendix S2: Statistical analysis of infection prevalence, infection intensity, and mouthpart loss

Matthijs Hollanders, Laura F. Grogan, Hamish I. McCallum, David A. Newell

To identify patterns in *Bd* infection status (infected/uninfected) and infection intensity (pathogen load), we fit a model to swab infection status and ($\log_{10}$) swab loads, respectively, correcting for measurement error in the qPCR and the sampling processes. Our model closely follows the model of DiRenzo *et al.* (2018), except that we used an informative prior for the sampling process measurement error, applied the Royle-Nichols (2003) model for pathogen detection probabilities, and further incorporated mouthpart loss status and intensity as descendant variables from *Bd* infection. Predictors were centered and scaled by two standard deviations to allow direct comparison between binary (site) and continuous (temperature and body size) effects (Gelman *et al.* 2008). We used reversible jump MCMC (RJMCMC, Green 1995) for predictor variable selection and constrained interaction effects to occur in the presence of respective main effects. We ran 4 chains for 50,000 iterations after discarding 10,000 as burn-in and thinning chains by 10, yielding 20,000 posterior samples. We summarised posterior distributions with medians and 95% highest posterior density intervals (HPDI) with RJMCMC inclusion probabilities, and considered effects important when 95% HPDIs of the coefficients did not overlap 0. We conducted posterior predictive checks (PPCs) for all four model components. The fully reproducible analysis is available at https://github.com/mhollanders/mfleayi-tadpoles.

Infection status

We modeled the latent true infection status (z_i) of tadpole $i \in 1, \dots, I = 865$ as a Bernoulli random variable:

$$z_i \sim \text{Bernoulli}\left(\psi_i\right) \tag{1}$$

where the individual probability of being infected with *Bd* (ψ_i) was modeled as a logit-linear function of covariates (site, temperature, log body size, and two/three-way interactions) and random survey effects. Note that 244 missing body size observations were imputed from the distribution of 621 observed values with MCMC.

The observed infection status (y_{ij}, data) during replicate qPCR run $j \in 1, 2$ was modeled—like an occupancy model—as a Bernoulli variable conditional on being infected ($z_i = 1$) and the probability of detecting *Bd* on the infected sample using qPCR (δ_i):

$$y_{ij} \sim \text{Bernoulli}\left(z_i \delta_i\right) \tag{2}$$

where δ_i was modeled as a function of swab infection intensity (n_i, see below), with r being the probability of detecting one $\log_{10}$ *Bd* gene copy on an infected sample in a qPCR run (Royle and Nichols 2003; Hollanders 2022):

$$\delta_i = 1 - (1 - r)^{n_i} \tag{3}$$

Infection intensity

We modeled the latent individual infection intensity (m_i) with a normal linear model:

$$m_i \sim \text{Normal}\left(\mu_i, \sigma_\mu^2\right) \tag{4}$$

where the expected infection intensity (μ_i) was modeled as a linear function of covariates (site, temperature, body size, and covariates) and random survey effects, and σ_μ is the population standard deviation. Random survey effects of infection status and intensity were modeled as draws from a bivariate normal distribution to explore potential correlations.

Knowing that there is measurement error associated with swab samples, but not having replicate samples to estimate this error for tadpoles, we relied on results from replicate samples collected from juveniles (Hollanders 2022) to estimate the sample infection intensity (n_i):

$$n_i \sim \text{Normal}\left(m_i, \sigma^2_{\text{swab}}\right) \tag{5}$$

where the measurement error of the swabbing process (σ_{swab}) was given an informative prior (see Table 1 in the main text and **Priors** below).

We modeled the observed infection intensity (x_{ij}, data) during replicate qPCR runs with a normal distribution centered on the sample infection intensity:

$$x_{ij} \sim \text{Normal}\left(n_i, \sigma^2_{\text{qPCR}}\right) \tag{6}$$

where σ_{qPCR} is the measurement error of the qPCR process.

Mouthpart loss

Next, we modeled the observed mouthpart loss status (w_i, data), as determined by the presence of dekeratinisation in either of the two jaw sheaths, as a Bernoulli variable:

$$w_i \sim \text{Bernoulli}\left(\lambda_i\right) \tag{7}$$

where the individual probability of having jaw sheath loss (λ_i) was modeled as a logit-linear function of estimated *Bd* infection status (z_i), *Bd* infection intensity (m_i), separate coefficients for body size with uninfected and infected tadpoles, and random survey effects (survey effects not shown):

$$\text{logit}\,\lambda_i = \alpha_\lambda + \beta_{\lambda_1} z_i + \beta_{\lambda_2} z_i \frac{m_i - \alpha_\mu}{2\sigma_\mu} + \beta_{\lambda_3} z_i \text{size}_i + \beta_{\lambda_4}\left(1 - z_i\right)\text{size}_i \tag{8}$$

Note that the effect of *Bd* infection intensity (β_{λ_2}) was only included for those individuals that were infected ($z_i = 1$), and that infection intensity was centered (by subtracting the average infection intensity α_μ) and scaled by two standard deviations $2\sigma_\mu$. This ensured that the interpretation of β_{λ_1} is the log odds change of having mouthpart loss due to an individual being

infected with *Bd* carrying the average infection intensity, and that β_{λ_2} was on the same scale as all other predictors.

Finally, we modeled the observed mouthpart loss intensity (v_i, data), quantified as the sum of the ordinal scores between 1–5 of both the top and bottom jaw sheaths for those individuals where mouthpart loss was detected, as an ordered probit regression with $s \in 1, \ldots, S = 10$ possible scores:

$$\begin{aligned} v_i &\sim \text{Categorical}\left(\kappa_{[1\ldots S]_i}\right) \\ \kappa_{[1]_i} &= \Phi\left(\tau_1 - \mu_{\kappa i}\right) \\ \kappa_{[2\ldots S-1]_i} &= \Phi\left(\tau_{[2:S-1]} - \mu_{\kappa i}\right) - \Phi\left(\tau_{[(2\ldots S-1)-1]} - \mu_{\kappa i}\right) \\ \kappa_{[S]_i} &= 1 - \Phi\left(\tau_{[S-1]} - \mu_{\kappa i}\right) \end{aligned} \tag{9}$$

where κ is a probability simplex (summing to 1) of length S, Φ is the cumulative standard normal distribution function (with standard deviation fixed at 1), τ is a vector of $S-1$ thresholds modeled as $\tau_s \sim \text{Normal}\left(\alpha_\tau + \beta_\tau\left(s - S/2\right), \sigma_\tau^2\right)$, and μ_{κ_i} is the mean of the standard normal modeled as a linear function of *Bd* infection intensity, body size, and random survey effects (not shown). Infection status was omitted from the model due to the lack of uninfected individuals with mouthpart loss and the resulting poor estimability of that parameter. Random survey effects of mouthpart loss status and intensity were also modeled as correlated using a bivariate normal distribution.

Priors

We used a mixture of vague, weakly informative, and informative priors (Table 1, main text). We specified a $\text{Beta}\left(3, 3\right)$ prior for the back-transformed intercept of *Bd* infection status and a weakly informative Student-t prior on the intercept of the log-linear function of infection intensity, centered on the observed average swab infection intensity. We used a conservatively informative $\text{Beta}\left(1, 10\right)$ prior on the probability of having mouthpart loss for uninfected tadpoles to reflect the low incidence in the sample (5%). We used weakly informative $t_3\left(0, 1\right)$ priors on all coefficients (with predictors standardised by two standard deviations, Gelman *et al.* 2008) to ensure some regularisation and improved MCMC mixing, while allowing for more extreme

coefficients. Similarly, we used $t_3^+(0,1)$ priors on standard deviation parameters, except for the measurement of the swabbing and qPCR processes. Since we did not collect replicate swab samples to estimate this error, we relied on previous work on replicate samples of juvenile *Mixophyes fleayi* from Brindle Creek, using that estimate for a $t_3^+(0.17, 0.11)$ prior (Hollanders 2022). Again, we applied a t prior as the thicker tails allow for deviating values. Although we did have data to estimate the measurement error of the qPCR process, we still used an informative $t_3^+(0.5, 0.05)$ prior because the previous work applied the same diagnostic protocol. We specified a somewhat informative $\text{Beta}(6,4)$ for the probability of detecting one $\log_{10}$ *Bd* gene copy with qPCR (Hollanders 2022). We applied $\text{LKJ}(2)$ priors on the Cholesky factors of the correlation matrices of correlated random survey effects. For the ordinal threshold parameters, we specified a $\text{Normal}(0,1)$ for the intercept τ_α and an $\text{Normal}^+(0,1)$ for τ_β to reflect the constraint that thresholds are ordered. We set a $\text{Beta}(1,1)$ prior for the RJMCMC inclusion probability.

Posterior predictive checks

We conducted PPCs for the *Bd* infection prevalence, infection intensity, and mouthpart loss components of the tadpole model. For each of 20,000 MCMC samples, we simulated replicate infection status (y^{rep}) and intensity (x^{rep}), and mouthpart loss (w^{rep}) and intensity (v^{rep}) datasets from the joint posterior distribution and computed fit statistics for both the observed data and replicate datasets.

For infection status, the binary response was not suitable for test statistics such as χ^2 or Freeman-Tukey statistics, and responses are usually binned across some useful categories as a solution (Kéry and Schaub 2012). In our model, we first summed infection status of individual i across duplicate qPCR run j (y_{ij} and y_{ij}^{rep}) and then binned results for each ($n = 25$) survey $t \in 1, \ldots, T = 25$, yielding y_{survey_t} and $y_{\text{survey}_t}^{\text{rep}}$. For the individual expected value (E_i), we used $2\psi_i\delta_i$ (to incorporate both the latent expected infection prevalence [ψ], the probability of detecting *Bd* [δ], and the number of qPCR runs [2]), which were also summed for each survey, yielding E_{survey_t}. The fit statistics used were Freeman-Tukey statistics calculated for each survey t, leading to the discrepancy measures (D_y and D_y^{rep}) calculated below:

$$
\begin{aligned}
D_y &= \sum_{t=1}^{25} \left(\sqrt{y_{\text{survey}_t}} - \sqrt{E_{\text{survey}_t}} \right)^2 \\
D_y^{\text{rep}} &= \sum_{t=1}^{25} \left(\sqrt{y_{\text{survey}_t}^{\text{rep}}} - \sqrt{E_{\text{survey}_t}} \right)^2
\end{aligned}
\tag{10}
$$

For infection intensity, we used χ^2 fit statistics on observed and replicated infection intensity of qPCR runs (x_{ij}) with μ_i as the expectation. The discrepancy measures were the fit statistics summed over all individuals and qPCR runs:

$$
\begin{aligned}
D_x &= \sum_{i=1}^{865} \sum_{j=1}^{2} \frac{\left(x_{ij} - \mu_i \right)^2}{\mu_i} \\
D_x^{\text{rep}} &= \sum_{i=1}^{865} \sum_{j=1}^{2} \frac{\left(x_{ij}^{\text{rep}} - \mu_i \right)^2}{\mu_i}
\end{aligned}
\tag{11}
$$

For mouthpart loss status, we also binned observed and replicated data, along with individual expected value λ_i, across surveys, and calculated Freeman-Tukey statistics, summing across surveys to yield the discrepancy measures:

$$
\begin{aligned}
D_w &= \sum_{t=1}^{25} \left(\sqrt{w_{\text{survey}_t}} - \sqrt{E_{\text{survey}_t}} \right)^2 \\
D_w^{\text{rep}} &= \sum_{t=1}^{25} \left(\sqrt{w_{\text{survey}_t}^{\text{rep}}} - \sqrt{E_{\text{survey}_t}} \right)^2
\end{aligned}
\tag{12}
$$

For mouthpart loss intensity, we used χ^2 statistics on observed and replicated intensity (v_i) with $\sum_{s=1}^{S} \kappa_{s_i} s$ as the expectation, which were subsequently summed over all individuals to arrive at the discrepancy measures:

$$
\begin{aligned}
D_v &= \sum_{i=1}^{865} \sum_{s=1}^{10} \frac{\left(v_i - \sum_{s=1}^{10} \kappa_{s_i} s \right)^2}{\sum_{s=1}^{10} \kappa_{s_i} s} \\
D_v^{\text{rep}} &= \sum_{i=1}^{865} \sum_{s=1}^{10} \frac{\left(v_i^{\text{rep}} - \sum_{s=1}^{10} \kappa_{s_i} s \right)^2}{\sum_{s=1}^{10} \kappa_{s_i} s}
\end{aligned}
\tag{13}
$$

We then visually inspected the discrepancies by plotting the discrepancy of the simulated datasets against the discrepancy of the observed dataset for all 20,000 MCMC samples (Figure S3), and

calculated the Bayesian p-values (BPVs) as the proportion of samples where the discrepancy of simulated data was greater than the discrepancy of the observed data ($\Pr(D^{\text{rep}} > D)$).

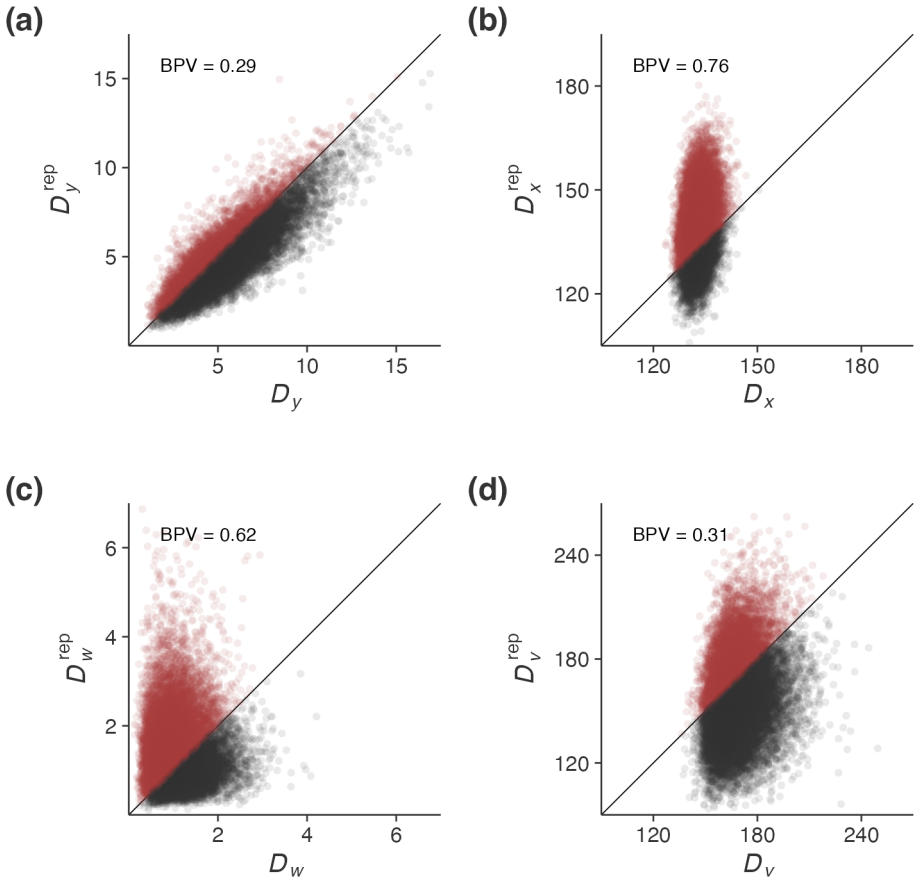

Figure S3: Discrepancy measures from simulated datasets (D^{rep}) versus observed data (D) for each of 20,000 MCMC samples for **(a)** *Bd* infection prevalence (calculated from Freeman-Tukey statistics), **(b)** *Bd* infection intensity (calculated from χ^2 statistics), **(c)** mouthpart loss status (calculated from Freeman-Tukey statistics), and **(d)** mouthpart loss intensity (calculated from χ^2 statistics). Bayesian p-values (BPVs) are the proportion of MCMC samples for which the discrepancy of replicated data was greater than the discrepancy of the observed data ($\Pr(D^{\text{rep}} > D)$). The color red indicates when $D^{\text{rep}} > D$.

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